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TITLE: High throughput sequencing of germline and tumor from men with early-onset, metastatic prostate cancer

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14. ABSTRACT

In this project, we proposed to us next generation sequencing approaches to analyze the germline (from blood and/or normal prostate tissue) and prostate cancer tissue from 20 men with early-onset, metastatic prostate cancer in order to identify germline variants that increase the risk of developing clinically significant prostate cancer, as well as novel driving somatic alterations. We have successfully, enrolled 9 men and collected germline DNA on 8 of those men and tumor samples on ?. Exome-sequencing of germline DNA has been performed and is currently being analyzed for 3 men. In the coming year, we plan to continue enrollment, tissue procurement, and sequencing as well as to begin the analysis of sequencing data and identification of genetic variants of interest. Given the uniqueness of the cohort in this project, we expect that novel driving genes with germline and/or somatic variants will be identified, which can be followed up with future functional studies.

15. SUBJECT TERMS

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INTRODUCTION

In this project, next generation sequencing (NGS) approaches will be used to analyze the germline (from blood and/or normal prostate tissue) and prostate cancer tissue from men with newly diagnosed Stage 4 (Tx N1 or M1) prostate cancer. All patients will have *de novo* metastatic disease diagnosed with prostate cancer at or before age 60 years. Men of European and African American descent will be included in this study. The goal of this project is to identify germline variants that increase the risk of developing clinically significant prostate cancer, as well as novel driving somatic alterations. We believe that men with early-onset, aggressive prostate cancer are more likely to harbor such variants.

KEYWORDS

Prostate cancer, germline, somatic, susceptibility, metastatic, early-onset

OVERALL PROJECT SUMMARY

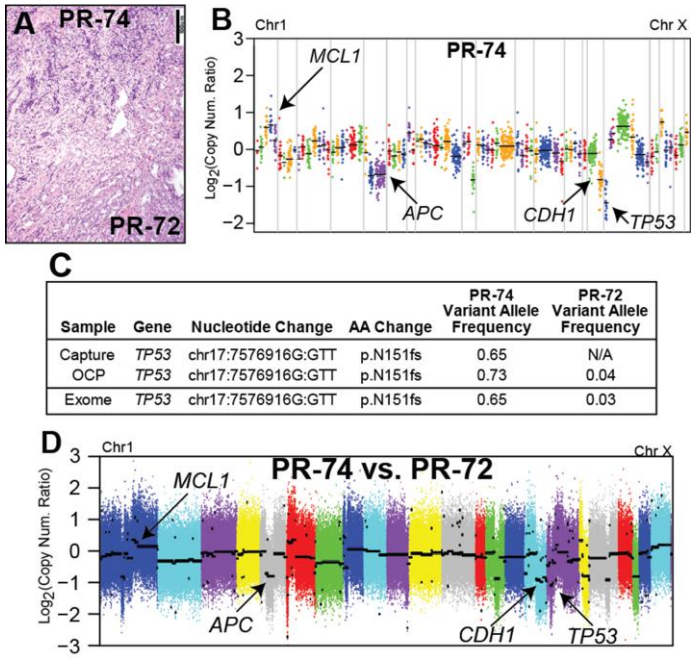
During the first year of funding, we have made significant progress on Major Task 1: identifying and enrolling men with de novo metastatic prostate cancer presenting at or before age 60 years into research project. We have finalized the IRB protocol and consent form, and received approval from both our local IRB and the DOD USAMRMC Office of Research Protections Human Research Protection Office. To facilitate the identification and recruitment of eligible participants, Dr. Cooney has notified clinicians in the University of Michigan (UM) Genitourinary Oncology Program and Department of Urology about the study and enrollment criteria. Additionally, we have developed a brochure and flyer (**Appendix A**) designed to be made available for patients in UM clinics describing the study and providing information about enrollment; these documents are awaiting IRB approval. To date, we have enrolled and consented 9 individuals.

Regarding Major Tasks 2 and 3, performing exome and/or transcriptome sequencing on germline DNA and prostate tumor DNA/RNA from 20 men with early-onset, stage 4 prostate cancer, whole-blood has been collected and germline DNA isolated from 8 of the 9 enrolled men, with the 9th pending. Whole-exome sequencing has been conducted on 3 germline samples thus far; data from those samples is currently being analyzed. In addition to whole-blood, paraffin-embedded prostate tumor samples have been collected for 4 of our 9 participants. Some of the participants have come from outside institutions so we have developed letters signed by Dr. Tomlins to request the original tumor biopsies. We are in the process of isolating DNA and RNA from the tumor tissue for NGS analysis.

In our work flow, we have discovered that few of our patients are identified before diagnostic procedures are performed which limits the opportunity to collect frozen specimens. Recognizing this challenge, Dr. Tomlins has continued to develop state of the art technologies to use formalin-fixed paraffin-embedded (FFPE) prostate cancer specimens for molecular studies to characterize the prostate cancer genome and transcriptome. These include: robust protocols for

co-isolating DNA/RNA from FFPE tissues, capture based NGS and qPCR approaches, and multiplexed PCR NGS based approaches. Additionally, this includes the ability to perform whole exome sequencing or WES on small amounts of FFPE prostate tumors. This is demonstrated in **Figure 1** which shows elegant characterization of adenocarcinoma and small cell carcinoma of the prostate arising in the same individual yet harboring different molecular events. The Tomlins laboratory has applied these same approaches to interrogate hundreds of routine FFPE cancer specimens on the Ion Torrent platform, including profiling over >100 FFPE prostate cancer specimens from all stages of disease. Dr. Tomlins has exploited the wealth of published and ongoing comprehensive profiling efforts to generate custom targeted panels that interrogate critical DNA alterations [genes with recurrent point mutations, indels or copy number alterations (CNAs)] and RNA alterations/pathways [androgen receptor (AR) signaling, *AR* splice variants, proliferation, neuroendocrine markers, gene fusions, subtyping genes, etc.]. Such approaches are critical to assess archived routine FFPE tumors from patients enrolled in the DOD IDEAS award proposal who may be identified months to years after their initial diagnosis, and lack fresh frozen tissue required for standard comprehensive molecular analysis.

Fig 1. Exome sequencing from FFPE tissues. **A**. Conventional (PR-72) and small cell (PR-74) prostate cancer components were isolated from the same FFPE tissue sections. **B**. NGS of PR-74 using the OCP identified copy number alterations in known drivers, including *TP53*. **C**. OCP capture-based NGS identified a deleterious *TP53* mutation exclusively in PR-74. Whole exome sequencing was also performed on both components using 1µg DNA (107x and 106x coverage using one P1 chip per sample), which confirmed the *TP53* mutation in PR-74. **D**. Copy number analysis from exome sequencing of PR-74 and PR-72 confirmed copy number alterations seen by OCP (compare to **B.**)



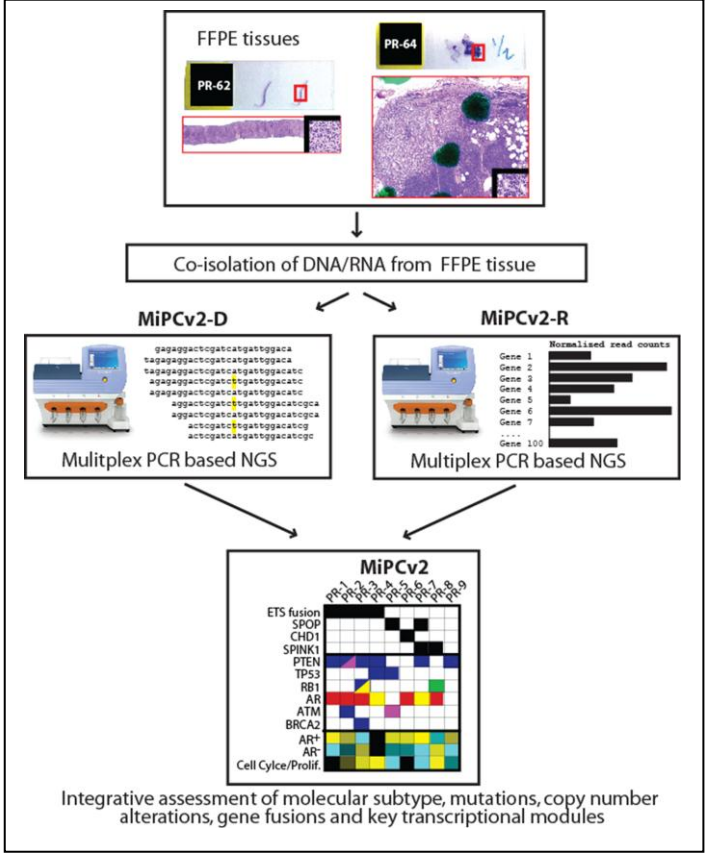
Sample	Gene	Nucleotide Change	AA Change	PR-74 Variant Allele Frequency	PR-72 Variant Allele Frequency
Capture	*TP53*	chr17:7576916G:GTT	p.N151fs	0.65	N/A
OCP	*TP53*	chr17:7576916G:GTT	p.N151fs	0.73	0.04
Exome	*TP53*	chr17:7576916G:GTT	p.N151fs	0.65	0.03

For the proposed studies of early-onset prostate cancer, we will now be relying on use of archived FFPE biopsy and prostatectomy tumor specimens. Using an optimized protocol, Dr. Tomlins' lab has co-isolated DNA and RNA from over 700 routine FFPE cancer specimens. For example, across 182 total prostate cancer specimens (32% biopsies, median 6 x 10um sections per specimen, ~1/2 requiring macrodissection to enrich for tumor content), we obtained a median of 1.1/2.8ug DNA/RNA. Importantly, from diagnostic biopsies (n=38) and RP (n=93)

specimens, we obtain a median of 20ng/171ng and 396ng/665ng DNA/RNA *per 10um section*, respectively.

For this proposal, we will therefore focus on using qPCR/target capture DNA based NGS approaches, as well as multiplexed PCR based RNA/DNA NGS approaches to interrogate the prostate cancer transcriptome/genome. The platform, referred to as MiPC v2, is outlined in **Fig 2**. Twenty ng of DNA is used for targeted multiplex PCR-based NGS for analysis of point mutations/indels and CNAs (**Fig 2, left**). Similarly, 20 ng of RNA is used for targeted multiplexed RT-PCR based NGS to assess fusion detection and gene expression profiling. Although we have chosen to use a targeted approach to analyze the prostate tumors, we consider expanding our approach in some cases to include whole exome sequencing. This strategy will be used if we identify tumors that lack clear known driver mutations (e.g. ETS fusion or *PTEN* deletion).

Fig 2 Schema for molecular characterization of prostate cancer samples (MiPC v.2).



KEY RESEARCH ACCOMPLISHMENTS Nothing to report

CONCLUSION

We have enrolled and begun genetic analyses on nearly half of the proposed 20 men with early-onset, metastatic prostate cancer. In the coming year, we plan to continue enrollment, tissue procurement, and sequencing as well as to begin the analysis of sequencing data and identification of genetic variants of interest. Given the uniqueness of the cohort in this project, we expect that novel driving genes with germline and/or somatic variants will be identified, which can be followed up with future functional studies.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS Nothing to report

INVENTIONS, PATENTS AND LICENSES Nothing to report

REPORTABLE OUTCOMES Nothing to report

OTHER ACHEIVEMENTS Nothing to report

REFERENCES None

APPENDIX A. Recruitment brochure and flyer.

About the Study

This study, officially titled "High Throughput Sequencing of Germline and Tumor from Men with Early-Onset Metastatic Prostate Cancer" will analyze blood, normal prostate tissue, and prostate cancer tissue from men with newly diagnosed Stage 4 prostate cancer at age 60 or younger.

We hope to identify genetic events that contribute to aggressive disease.

Men of all racial and ethnic backgrounds will be included in the study.

Eligibility Questions?

Study Coordinator
Prostate Cancer Genetics Project– DOD
7436 CC, 1500 E. Medical Center Drive
Ann Arbor, MI 48109

734-936-2031 (phone)
734-647-4338 (fax)
Pcgp-project@med.umich.edu

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MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

High Throughput Sequencing of Germline and Tumor from Men with

Early-Onset Metastatic

Prostate Cancer

University of Michigan

Comprehensive Cancer Center

WHO CAN HELP?

Men with newly diagnosed advanced prostate cancer (T4 and/or N1 and/or M1) at age 60 or younger who are

- Undergoing standard of care surgeries or procedures

OR

- Have prostate cancer tissue available for molecular evaluation

WHAT IS INVOLVED?

- Signing a consent form
- Completing a basic questionnaire
- Providing a small blood sample

Traveling to Ann Arbor is not required.

Participation is completely voluntary and confidential and involves no cost, and the decision to participate in no way affects treatment.

Why is this important?

Prostate cancer is the *second leading cause of cancer deaths* in men the U.S.

Early-onset prostate cancer in a family member has been shown to increase risk of prostate cancer in other family members.

Individuals diagnosed with early-onset cancers are much more likely to have a genetic component to their disease.

Volunteer Today!

For eligibility questions, contact
Study Coordinator
734-936-2031 (phone)
734-647-4338 (fax)
Pcgp-project@med.umich.edu

For clinical questions, contact
Kathleen A. Cooney, MD
kcooney@med.umich.edu

Scott Tomlins, MD, PhD
tomlinss@med.umich.edu

MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

HIGH THROUGHPUT SEQUENCING OF GERMLINE AND TUMOR FROM MEN WITH EARLY-ONSET, METASTATIC PROSTATE CANCER

This study will analyze blood, normal prostate tissue, and prostate cancer tissue from men with newly diagnosed Stage 4 prostate cancer to identify genetic events that contribute to aggressive disease.

Who can help?

Men with newly diagnosed advanced prostate cancer (T4 and/or N1 and/or M1) at age 60 or younger who are:

- Undergoing standard of care surgeries or procedures
  OR
- Have prostate cancer tissue available for molecular evaluation

What is involved?

- Signing a consent form
- Completing a basic questionnaire
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For clinical questions contact:

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University of Michigan
Medical School

This generic Statement of Work document is intended to assist applicants with the format preferred by CDMRP. This particular SOW does not contain any specific scientific information and is intended to be easily modifiable for any project. Not all components will be applicable for every project; please consult your Program Announcement for specific award requirements.

STATEMENT OF WORK – Month/Day/Year
PROPOSED START DATE Sept. 01, 2013

Site 1: University of Michigan
3003 S. State Street
Ann Arbor, MI 48109-1274
PI: Kathleen Cooney, MD

Site 2: University of Carolina – Chapel Hill
104 Airport Drive, Suite 2200
Chapel Hill, NC 275-7264
Partnering PI: Ethan Lange, PhD

Specific Aim 1(specified in proposal)	Timeline	Site 1	Site 2
Major Task 1: To identify and enroll men with de novo metastatic prostate cancer presenting at or before age 60 years into research project	**Months** 1-27	University of Michigan	North Carolina
Subtask 1 A. Finalize IRB protocol and consent form; this process has been initiated and we expect that documents will be completed shortly after study initiation	1	Kathleen Cooney, MD	
Subtask 1 B. Initiate process to notify all clinicians in UM Genitourinaty Oncology Program about study and identify potential participants	1-3	Kathleen Cooney, MD and Scott Tomlins MD/PhD	
Subtask 1 C. Complete IRB approval process	2-3	Kathleen Cooney, MD	
Subtask 1 D. Recruit participants to study	3-27	Kathleen Cooney, MD and Scott Tomlins MD/PhD	
Milestone(s) Achieved			
Local IRB/IACUC Approval	3		
Milestone Achieved: HRPO/ACURO Approval			
Major Task 2: To perform exome sequencing on germline DNA samples from 20 men with early-onset Stage 4 prostate cancer.	**Months** 3-36	University of Michigan	North Carolina
Subtask 2 A. Collect whole blood from 20 eligible participants and isolate DNA from leukocytes	3-27	Kathleen Cooney, MD	

		(n=20)	
Subtask 2 B. Complete exome sequencing, alignment and variant calling	3-30	Scott Tomlins, MD/PhD	
Subtask 2 C. Prioritize variants for followup	13-30	Kathleen Cooney, MD and Scott Tomlins MD/PhD	Ethan Lange, PhD
Subtask 2 D.Genotype top candidate variants in prostate cancer cases (~5000 from UM and Johns Hopkins) and controls (~1500 men without prostate cancer from Johns Hopkins University)	18-36	Kathleen Cooney, MD	
Milestone(s) Achieved:			
Major Task 3: To conduct exome/transcriptome analyses on prostate cancer tumor from 20 study participants with metastatic prostate cancer diagnosed at or before age 60.	**Months** 3-36	**University of Michigan**	**North Carolina**
Subtask 3 A. Collect sample(s) of prostate cancer from 20 study participants. For those men who are having a surgical procedure, extra tumor not required for clinical purposes will be used. For the remaining participants, formalin-fixed paraffin-embedded (FPPE) tumor will be obtained from prior diagnostic or therapeutic procedures	3-27	Kathleen Cooney, MD and Scott Tomlins MD/PhD	
Subtask 3 Complete tumor exome and transcriptome sequencing, including analysis of copy number, gene-fusions, and outliers	3-30	Kathleen Cooney, MD and Scott Tomlins MD/PhD	Ethan Lange, PhD
Subtask 3 Prioritize potential candidate mutations for follow up using driver analysis pipeline	13-36	Kathleen Cooney, MD and Scott Tomlins MD/PhD	Ethan Lange, PhD
Subtask 3 Test proposed driver mutations in a set of 100 FPPE prostate cancer specimens	18-36	Scott Tomlins, MD/PhD	

If human subjects are involved in the proposed study, please provide the projected quarterly enrollment in the following table.

	Year 1				**Year 2**				**Year 3**
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**
Site 1	3	2	3	2	3	2	3	2	
Target Enrollment (cumulative)	**3**	**5**	**8**	**10**	**13**	**15**	**18**	**20**	
									No Subject Recruitment – Follow up only

Note: The Government reserves the right to request a revised SOW format and/or additional information.